

(19)



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(11)

**EP 0 821 055 B1**

(12)

**EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention  
of the grant of the patent:  
**03.12.2003 Bulletin 2003/49**

(51) Int Cl.7: **C12G 3/06**, C12C 5/02,  
A23L 1/236, A23L 1/226,  
A23L 1/38

(21) Application number: **97304614.7**

(22) Date of filing: **27.06.1997**

**(54) Use of monellin in enhancing the flavour of beverages and foodstuffs**

Verwendung von Monellin zur Erhöhung des Aromas von Getränken oder Nahrungsmitteln

Utilisation de monelline pour fortifier l'arôme de boissons et aliments

(84) Designated Contracting States:  
**DE FR GB IT**

(30) Priority: **25.07.1996 US 22597 P**  
**06.03.1997 US 813884**

(43) Date of publication of application:  
**28.01.1998 Bulletin 1998/05**

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**EP 0 821 055 B1**

## Description

[0001] The use of monellin in an amount below the sweetness threshold in order to enhance the flavour of various oral compositions is known from US-A-4 412 984.

5 [0002] Our invention is directed to fermentation-derived beverages and distilled spirits which are consumable or ingestible and which comprise:

(i) from 1.5% up to 70% by weight of an alcohol or ester selected from the group consisting of ethyl alcohol, isoamyl alcohol, *n*-hexanol, *L*-menthol and *cis*-3-hexenyl acetate;

10 (ii) from 0.5 parts per million (ppm) up to 15 ppm of monellin or an analogue thereof; and

(iii) from about 0.1 parts per billion (ppb) up to about 2% by weight of a fermentation-derived beverage or distilled spirit flavorant.

15 [0003] Our invention is also directed to a process for augmenting or enhancing the aroma and/or taste of a fermentation-derived beverage or distilled spirit comprising the step of adding to a fermentation-derived beverage or distilled spirit from 0.5 ppm up to 15 ppm of monellin or an analogue thereof.

20 [0004] Also disclosed are monellin (or an analogue thereof) containing flavourants containing from about 0.5 up to about 15ppm of monellin or an analogue thereof and from about 0.01 up to about 200 ppm of at least one flavorant selected from the group consisting of:

isobutyl thiazole;  
 trimethyl pyrazine;  
 25 2-methoxy-3-isobutyl pyrazine;  
 menthol;  
 isopulegol;  
 trans-2-hexenol;  
 6-methyl-5-hepten-2-one;  
 30 methyl acetophenone;  
 vanilla;  
 vanillin;  
 3-methyl-2-buten-1-ol;  
 a mixture of 3-methyl-2-buten-1-ol and ethyl acetate;  
 35 a mixture of 3-methyl-2-buten-1-ol, ethyl acetate and CITRAL ;  
 natural banana essence;  
 ethyl decanoate;  
 natural raspberry flavor essence in admixture with  $\alpha$ -terpineol;  
 $\beta$ -ionone;  
 40 bis(methylfuryl)disulfide;  
 farnesene;  
 germacrene;  
 ethyl pyruvate;  
 1,3,5-undecatriene; and  
 45 indole.

[0005] Monellin (or an analogue thereof) in admixture with peanut butter, preferably low-fat peanut butter, is also disclosed.

50 [0006] As will be seen in the examples provided, *infra*, the monellin or analogue thereof has an extremely favorable flavor and mouthfeel impact upon the fermentation-derived beverage or distilled spirit. Examples of fermentation-derived beverages and distilled spirits are beer (both low alcohol beer, medium alcohol beer and high alcohol beer), wine, liqueurs; and hard liquors including vodka, rum, brandy, bourbon, Scotch whiskies and the like.

[0007] The term "monellin or an analogue thereof" can mean natural monellin protein such as that disclosed by Somoza, et al, in *J. Mol. Biol.* (1993), 234, pages 390-404, title "Two Crystal Structures of a Potently Sweet Protein/ Natural Monellin at 2.75 Å Resolution and Single-Chain Monellin at 1.7 Å Resolution" defined according to Figures 1, 2 and 4B described, *supra*, or analogues thereof as set forth in the following list:

TABLE I

## ANALOGUES OF NATIVE MONELLIN

Monellin analogue
Native monellin of Figures 1, 2 and 4B
GluB49Asn, AsnB50Glu
AspA22Asn, GluA25Gln, AspA26Asn, GluB49Asn, AsnB50Glu
GluB49Asn, AsnB50Glu
CysB41Ser
AspA22Asn
GluA25Gln
AspA26Asn
AspB7Asn
AspB7Abu (Abu = L- $\alpha$ -aminobutyric acid)
AspB7Glu
AspB7Gly
AspB7D-Asp
IleB6Gly
IleB6Ala
IleB6D-Ala
IleB8Gly
IleB8Ala
IleB8Phe
IleB8D-Ala
TyrA13Gly
TyrA13Phe
GlyB9Ala
GlyB9D-Ala
LysA4Ahx (Ahx = L- $\alpha$ -amino hexanoic acid)
LysA17Ahx
LysA28Ahx
LysA33Ahx
LysB17Ahx
LysB25Ahx
LysB36Ahx
GlnB13Ahx
LysB44Ahx
GlnB13Ahx, LysB55Ahx

[0008] In addition, the "monellin or an analogue thereof" of our invention can be a single-chain monellin or mutant thereof as set forth in Figure 3A or Figure 3B(ii) and prepared according to Published Japanese Application No. JP05/070494 (abstracted at *Chemical Abstracts*, Volume 119:48069g, thusly:

119: 48069g Single-stranded monellins for improved thermostability. Iijima, Hiroshi; Sone, Hidetaka (Kirin Brewery) Jpn. Kokai Tokkyo Koho JP 05 70,494 [93 70,494] (Cl. C07K7/10), 23 Mar 1993, JP Appl. 90/196,983, 25 Jul 1990; 29 pp. The single-stranded (ss) monellins (I) retain the sweetness of the wild type two-stranded I and have enhanced thermostability, esp. in lower pH, and protease resistance. The ss-I are prep'd. by linking the C-terminus of B chain of I with the N-terminal Phe of A chain with a linker amino acid Xaa (Xaa = Gly, Ser, Glu, or Thr). Prepn. of a ss-I using Gly as the linker amino acid by expression of the synthetic gene in *Escherichia coli* and *Saccharomyces cerevisiae* was shown. The ss-I retained its sweetness after incubation for 20 mins at 100°, but the wild type lost its sweetness at 50°. Moreover, the ss-I but not the wild type counterpart was resistant to proteinase degrad. with e.g. *Achromobacter* proteinase I. Replacing the Val<sub>12</sub> or Val<sub>37</sub> of the ss-I with Ala did not alter the thermostability and protease resistance. or according to the procedure of U.S. Letters Patent No. 5,478,923 issued on December 26, 1995. Examples of mutants of single-chain monellin are as follows:

TABLE II

SINGLE-CHAIN MONELLIN MUTANTS

Single-chain monellin of Figure 3A
Single-chain monellin of Figure 3B (ii)
Lys43Glu
Arg70Glu
Lys17Cys, Phe34Cys (disulfide)
Lys44Cys, Glu57Cys (disulfide)
Lys44Gln, Glu48Gln, Glu50Gln, Arg57Gln, Glu52Gln, Lys54Gln
Gln59Cys, Asp62Cys, Cys41Ser
Phe34Asp
Gly1Met, Glu2Met
Glu59Cys
Cys41Ser
Met42Val
Trp3Thr, Ile5Glu, Met42Asn
Deletion of Pro94
Deletion of Pro92 to Pro94
Deletion of Pro90 to Pro94

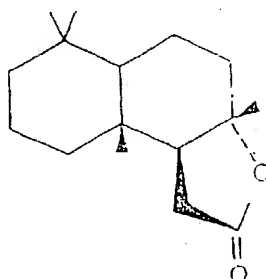
As stated above, the amount of monellin or analogue thereof may be used in the beverage at levels of from 0.05 ppm up to 15 ppm depending upon the nature of the beverage and the type of flavorant used. Indeed, good results are obtained as will be shown in the Examples, infra, at levels of 0.1 ppm with chocolate flavor using natural monellin or recombinant monellin. When used with cis-3-hexenyl acetate, levels of monellin or analogues thereof of from 3 and 5 ppm are useful. When used with liqueurs such as blueberry liqueur or Liqueur Perle de Brillet, levels of 10 ppm are useful.

[0009] In addition to the monellin or analogue thereof and flavor added to the alcoholic beverage composition, other materials have been found by us to be useful in augmenting or enhancing the aroma and taste of the fermentation-derived beverage or distilled spirit. These other materials are as follows:

(i) thaumatin, including Thaumatin I, Thaumatin II, Thaumatin B and mixtures thereof such as TALIN® (trademark of Tate and Lyle Limited of the United Kingdom);

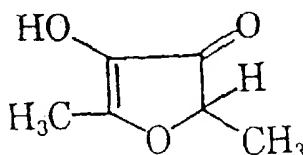
(ii) succinic acid;

(iii) sclareolide having the structure:



and

(iv) 2, 5-dimethyl-4-hydroxy-3(2H)-furanone having the structure:



The amount of succinic acid that can be used can vary from 0.05 ppm up to 2%. The amount of sclareolide that can be used may vary from 0.05 ppm up to 2%. The amount of thaumatin that can be used may vary from 0.1 ppb up to about 15 ppm by weight of the fermentation-derived beverage or distilled spirit.

[0010] When thaumatin is used with the monellin or analogue thereof, it is preferable to use a ratio of thaumatin: monellin or analogue thereof of about 1:1. However, for the purposes of our invention, a ratio of from about 0.05 parts monellin:1 part thaumatin up to 1 part thaumatin:0.05 parts monellin is within the scope of our invention.

[0011] The following examples are given to illustrate embodiments of the invention as it is preferred to practice it. It will be understood that these examples are illustrative and the invention is not to be considered as restricted thereto except as indicated in the appended claims.

#### EXAMPLE I

#### EXAMPLE I(A)

#### CHOCOLATE FLAVOR FORMULATION

[0012] The following formulation is prepared:

"AAA COMPOSITION"	
Ingredients	Parts by Weight
Sclareolide	50
Caryophyllene beta	50
5-Methyl-2-phenyl-2-hexanal	130

(continued)

<b>"AAA COMPOSITION"</b>	
<b>Ingredients</b>	<b>Parts by Weight</b>
Isovaleric acid	25
Phenyl acetaldehyde	80
Phenyl ethyl acetate	10
Geranyl acetate	30
Linalool	150
Furaneol 15%	240
2-Ethyl-3-methyl pyrazine	0.4
2-Ethyl-5-methyl pyrazine	0.1
2,3,5-Trimethyl pyrazine	0.4
Propylene glycol	234.1
<b>Total:1,000</b>	

**EXAMPLE I(B)**

[0013]

<b>COMPARISONS</b>		
<b>Water</b>	<b>Natural Monellin (1 ppm) of Figures 1, 2 and 4B</b>	<b>Recombinant Monellin of Figure 4A (1 ppm)</b>
<b>AAA 1 ppm:</b> Typical AAA (chocolate) note.	<b>AAA 1 ppm:</b> Typical AAA chocolate note with a larger mouthfeel. There is also a metallic/acid aftertaste in the back. The monellin seems to boost this type of aftertaste. <b>Ranking (on a scale of 1-10 with 10 being the best) : 7/10</b>	<b>AAA 1 ppm:</b> Typical AAA chocolate note, but here the metallic/acid aftertaste is reduced. <b>Ranking (on a scale of 1-10 with 10 being the best):9/10</b>

[0014] The water has now been replaced with filtered water in order to reduce the metallic/acid aftertaste and the results are as follows:

<b>Water</b>	<b>Monellin (1 ppm)</b>	<b>Recombinant Monellin of Figure 4A (1 ppm)</b>
<b>AAA 1 ppm:</b> Typical AAA note with a bitter/acid aggression on the back of the tongue.	<b>AAA 1 ppm:</b> Typical AAA note with a move of the bitter/acid impact to the front of the tongue. <b>Ranking (on a scale of 1-10 with 10 being the best) : 6/10</b>	<b>AAA 1 ppm:</b> Typical AAA note, but no more aggressivity of the bitter/acid impact. Something full which is filling up the mouth with a coating effect on the tongue. <b>Ranking (on a scale of 1-10 with 10 being the best) :9/10</b>

[0015] The chocolate flavor of Example IA is now used at a level of 0.1 ppm in 70% aqueous ethyl alcohol:

Water	Natural Monellin (1 ppm) of Figures 1, 2 and 4B	Recombinant Monellin of Figure 3B(ii) (1 ppm)
AAA 0.1 ppm (10 drops of alcohol solution) : Typical AAA note.	AAA 0.1 ppm (10 drops of ethanol solution) : Typical AAA, no difference in the flavor but enhancement of ethyl alcohol traces. Ranking (on a scale of 1-10 with 10 being the best):7/10	AAA 1 ppm (10 drops of ethanol solution) : Typical AAA note, but alcohol is stronger. Ranking (on a scale of 1-10 with 10 being the best):8/10

**EXAMPLE II****EXAMPLE II(A)**

[0016] The following mint flavor formulation is prepared:

Ingredients	Parts by Weight
Sclareolide	50
Caryophyllene beta	50
Methanone	50
Limonene	25
Thymol	20
Eucalyptol	10
Terpineol 4	4
Myrcene	4
para Cymene	2
gamma Terpinene	2
Peppermint oil	783
Total:1,000	

**EXAMPLE II(B)****COMPARISONS**

[0017]

Water	Natural Monellin (1 ppm) of Figures 1, 2 and 4B	Recombinant Monellin of Figure 4A (1 ppm)
Mint flavor of Example II(A) (0.1 ppm) : Typical mint flavor impact.	Mint flavor of Example II(A) at 0.1 ppm: Mint impact is depressed with ethyl alcohol impression covering the mint note. Ranking:6/10	Mint flavor of Example II(A) (1 ppm): Typical mint impact with a sweet alcohol note. The product is rounder. Ranking:8/10

**EXAMPLE III**

[0018] Additional comparisons were carried out as follows:

Water	Natural Monellin (10 ppm) of Figures 1, 2 and 4B	Recombinant Monellin of Figure 3B(ii) (10 ppm)
AAA flavor of Example I(A) (0.5 ppm) : Typical AAA (chocolate) note.	AAA flavor of Example I(A) (0.5 ppm) : Typical AAA note with a depression of the floral chocolate impact. The sweet impact is instantaneous with no delay. <b>Ranking:8/10</b>	AAA flavor of Example I(A) (0.5 ppm) : Typical AAA (chocolate) note; better flavor impact with alcohol impression. The sweet nuance is instantaneous, but with a small licorice aftertaste. <b>Ranking:9/10</b>

Water	Natural Monellin (10 ppm) of Figures 1, 2 and 4B	Recombinant Monellin of Figure 4A (10 ppm)
Isoamyl alcohol (1 ppm) : Typical isoamyl alcohol impression, fruity, winey.	Isoamyl alcohol (1 ppm) : Typical, but we have a stronger isoamyl alcohol impact together with a sweet aftertaste. <b>Ranking:8/10</b>	Isoamyl alcohol (1 ppm) : Typical, but much stronger isoamyl alcohol impact. Isoamyl alcohol is around your mouth with a coating impression. <b>Ranking:9/10</b>

Water	Natural Monellin (10 ppm) of Figures 1, 2 and 4B	Recombinant Monellin of Figure 4A (10 ppm)
Hexanol (1 ppm) : Typical hexanol note with a green, skinny, fruity, winey note.	Hexanol (1 ppm) : Typical stronger, winey hexanol note with a sweet aftertaste. <b>Ranking:8/10</b>	Hexanol (1 ppm) : Typical, but much stronger with a vegetable, full, strong, green note with a sweet impact. <b>Ranking:9/10</b>

Water	Natural Monellin (10 ppm) of Figures 1, 2 and 4B	Recombinant Monellin of Figure 4A (10 ppm)
Hexanol (5 ppm) : Typical hexanol note with a waxy, fatty, green beans note; the profile is flat.	Hexanol (5 ppm) : Typical stronger with a dry impression of rum. <b>Ranking:7/10</b>	Hexanol (5 ppm) : Typical, but much stronger with a nice, sweet impact. <b>Ranking:9/10</b>

Water	Natural Monellin (10 ppm) of Figures 1, 2 and 4B	Recombinant Monellin of Figure 4A (10 ppm)
Cis-3-hexenylacetate (5 ppm) : Typical green, fruity, beany tea note.	Cis-3-hexenyl-acetate (5 ppm): Typical stronger with a more green, living note with a sweet impact. <b>Ranking:9/10</b>	Cis-3-hexenyl-acetate (5 ppm) : Typical, but much stronger with a natural, full, strong, green note. The dosage is too strong. <b>Ranking:8/10</b>

Water	Natural Monellin (10 ppm) of Figures 1, 2 and 4B	Recombinant Monellin of Figure 4A (10 ppm)
Cis-3-hexenylacetate (3 ppm): Typical green, fruity, straw- berry note.	Cis-3-hexenyl-acetate (3 ppm) : Typical stronger with a more fresh green, living note. <b>Ranking: 7/10</b>	Cis-3-hexenylacetate (3 ppm): Typical, but no more chemical impact. <b>Ranking:9/10</b>

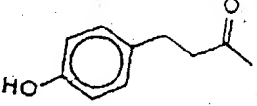


Water	Natural Monellin (10 ppm) of Figures 1, 2 and 4B	Recombinant Monellin of Figure 4A (10 ppm)
L-Menthol (5 ppm): Typical green, fruity, strawberry note.	L-Menthol (5 ppm): Typical stronger with a more fresh green, living note. Ranking:8/10	L-Menthol (5 ppm): Typical stronger. Ranking:9/10

Water	Natural Monellin (10 ppm) of Figures 1, 2 and 4B	Recombinant Monellin of Figure 4A (10 ppm)
Citral (0.5 ppm): Typical citral, lemon note.	Citral (0.5 ppm): Depression of citral, lemon impact. Ranking:4/10	Citral (0.5 ppm): Depression of the typical citral note. Ranking:4/10

Water	Natural Monellin (10 ppm) of Figures 1, 2 and 4B	Recombinant Monellin of Figure 4A (10 ppm)
Citral (5 ppm): Typical citral, lemon note.	Citral (5 ppm):Very strong depression of the citral, lemon impact. Ranking:4/10	Citral (5 ppm):Very strong depression of the typical citral note. Ranking:4/10

Water	Natural Monellin (10 ppm) of Figures 1, 2 and 4B	Recombinant Monellin of Figure 4A (10 ppm)
Gamma decalactone (5 ppm) : Typical butter lactone.	Gamma decalactone (5 ppm) : Butter lactone with a pungent taste and with a linoleic taste. Ranking: 5/10	Gamma decalactone (5 ppm) : Butter lactone with a fruity impression. Ranking:7/10

Water	Natural Monellin (10 ppm) of Figures 1, 2 and 4B	Recombinant Monellin of Figure 4A (10 ppm)
Oxyphenylon (15 ppm) : Sweet, warm, fruity raspberry, powdery taste. (Oxyphenylon has the structure: 	Oxyphenylon (15 ppm) : Boosting the raspberry flavor, but with a strong, soapy, powder note. Ranking:4/10	Oxyphenylon (15 ppm) : Direct impact of oxyphenylon notes, but the soapy note is very strong?? Ranking:3/10

Water	Natural Monellin (10 ppm) of Figures 1, 2 and 4B	Recombinant Monellin of Figure 4A (10 ppm)
Acetaldehyde (5 ppm) : Acetaldehyde impact.	Acetaldehyde (5 ppm): No working depressing the acetaldehyde impact. Ranking:2/10	Acetaldehyde (5 ppm) : Noworking depressing the acetaldehyde impact. Ranking:3/10

<b>Blueberry liquor (De Kuyper)</b>	<b>Blueberry liquor plus Natural Monellin (10 ppm) of Figures 1, 2 and 4B</b>	<b>Blueberry liquor plus Recombinant Monellin of Figure 4A (10 ppm)</b>
<i>Standard product with blueberry flavor plus extract.</i>	<i>More fruity, more volatility; the alcohol impact is stronger. Ranking: 7/10</i>	<i>The fruity impact is more ripe type, but the alcohol impact is very high. Ranking: 9/10</i>

<b>Blueberry liquor (De Kuyper)</b>	<b>Blueberry liquor plus "Thaumatococcus" (TALIN®) (0.5 ppm)</b>	<b>Blueberry liquor plus Natural Monellin (10 ppm) of Figures 1, 2 and 4B</b>	<b>Blueberry liquor plus Recombinant Monellin of Figure 4A (10 ppm)</b>
<i>Standard product.</i>	<i>More natural fruit flavor; no action on the alcohol level. Ranking: 6/10</i>	<i>Fruity with strong alcohol impact. Ranking: 7/10</i>	<i>Fruity, but very strong alcohol impact. Ranking: 8/10</i>

<b>Liqueur Perle de Brillet 24° Alc</b>	<b>Liqueur Perle de Brillet plus "Thaumatococcus" (TALIN®) (0.5 ppm)</b>	<b>Liqueur Perle de Brillet plus Natural Monellin (10 ppm) of Figures 1, 2 and 4B</b>	<b>Liqueur Perle de Brillet plus Recombinant Monellin of Figure 4A (10 ppm)</b>
<i>Apple Pear type of flavor.</i>	<i>More natural fruit flavor; no action on the alcohol level. Ranking: 6/10</i>	<i>More body; amyl alcohol and hexanol is higher with strong ethyl alcohol impact, 20% more. Ranking: 7/10</i>	<i>Fruity, but very strong alcohol impact may be 20-25% stronger. Ranking: 8/10</i>

<b>Neutral liquor</b>	<b>Neutral liquor plus Natural Monellin (5 ppm) of Figures 1, 2 and 4B</b>	<b>Neutral liquor plus Recombinant Monellin of Figure 4A (5 ppm)</b>
<i>Standard neutral product.</i>	<i>More fruity, more volatility; the alcohol impact is stronger. Ranking: 7/10</i>	<i>The alcohol impact is very high. Ranking: 9/10</i>

<b>Liquor 12° brix 20% alcohol with Lemon flavor (40 ppm)</b>	<b>Liquor 12° brix 20% alcohol with Lemon flavor (40 ppm) plus Natural Monellin (5 ppm) of Figures 1, 2 and 4B</b>	<b>Liquor 12° brix 20% alcohol with Lemon flavor (40 ppm) plus "Thaumatococcus" (TALIN®) (1 ppm)</b>	<b>Liquor 12° brix 20% alcohol with Lemon flavor (40 ppm) plus "Thaumatococcus" (TALIN®) (1 ppm) plus Recombinant Monellin of Figure 4A (1 ppm)</b>
<i>Lemon impact.</i>	<i>Stronger ethyl alcohol impact, depression of the lemon impact. Ranking: 5/10</i>	<i>The citrus impact is better, stronger, more natural, no impact on alcohol. Ranking: 6/10</i>	<i>Nice citrus impact with a stronger alcohol impression. Ranking: 8/10</i>

[0019] The lemon flavor of the foregoing Example III has the following formulation:

## EP 0 821 055 B1

Lemon Flavor	
Ingredients	Parts by Weight
Lemon essential oil	13.0
Citral	2.0
Cis-3-hexenyl acetate	1.0
Citric acid	2.0

[0020] The "apple pear type of flavor" of the foregoing Example III contains the following ingredients and parts by weight:

Pear Flavor	
Ingredients	Parts by Weight
Amyl acetate	1,340.0
Amyl valerate	130.0
Benzyl acetate (10% solution in 95% food grade ethyl alcohol)	25.0
Benzyl butyrate	1.0
Bergamot essential oil	1.0
<i>n</i> -Butyl acetate	1.0
Ethyl acetate	80.0
Ethyl butyrate	13.0
Ethyl decanoate	2.0
Ethyl heptylate	2.0
Ethyl hexylate	1.0
Ethyl octanoate	2.0
Geranyl propionate	100.0
Hexyl acetate	2.0
$\alpha$ -Ionone (1% solution in 95% food grade ethyl alcohol)	1.0
Isoamyl acetate	1.0
Isoamyl valerate	1.0
CITRAL™	3.0
Methyl acetate	1.0
Methyl heptenone	1.0
Orris resinoid (1% solution in 95% food grade ethyl alcohol)	5.0
Propyl acetate	1.0
Citronellol (1% solution in 95% food grade ethyl alcohol)	1.0
$\gamma$ -Undecalactone	1.0
Vanillin	5.0
Propylene glycol	279.0
Total	2,000.0

[0021] The blueberry flavor used in the foregoing Example III has the following formulation:

<b>Blueberry Flavor</b>	
<b>Ingredients</b>	<b>Parts by Weight</b>
Amyl acetate	500.0
Amyl butyrate	15.0
Anethol	0.5
Benzyl salicylate	10.0
Acetyl methyl carbinol	8.0
Dimethyl anthranilate	1.0
Ethyl acetate	60.0
Ethyl butyrate	74.0
Ethyl methylphenylglycidate	20.0
Ethyl succinate	1.0
Ethyl valerate	1.0
Geraniol	13.0
Hexanal	1.0
Cis-3-hexen-1-ol	1.0
2-Hexenyl acetate	1.0
2-Hexenyl butyrate	1.0
Hexyl butyrate	1.0
4-( <i>p</i> -Hydroxyphenyl-2-butanone)	16.0
$\alpha$ -Ionone	210.0
Isobutyl cinnamate	1.0
Jasmine, absolute (10% solution in 95% food grade ethanol)	7.0
Lemon essential oil	13.0
Maltol	3.0
Methyl butyrate	1.0
Methyl capronate	1.0
Methyl disulfide	1.0
methyl <i>p</i> -naphthyl ketone	1.0
50-50 Weight-weight mixture of orris resinoid and $\alpha$ -irone)	60.5
Rose, absolute	10.0
Terpenyl acetate	1.0
$\gamma$ -Undecalactone	10.0
Ethyl vanillin	86.0
Propylene glycol	870.0
Total	2,000.0

**EXAMPLE IV****FLAVORANTS AND PEANUT BUTTER CONTAINING, IN ADDITION, NATURAL MONELLIN**

5 [0022] The following table sets forth various flavorants used at various levels in aqueous solutions of ethanol, their evaluations and the ranking of same on a scale of 1-5, with 5 being the best of the overall sensory perception value thereof when using 5 ppm of natural monellin of Figures 1, 2 and 4B.

10	Molecule	Molecule Concentration	Evaluation	Quality Ranking on a Scale of 1-5, with 5 Being the Best of Overall Sensory Perception Value
15	Natural dimethyl sulfide	2 ppm (using a 1% solution)	The monellin decreases volatility, but causes a larger flavor impact. Also a "non-sugar" sweetness impact is created.	2
20	Ethyl acetate	5 ppm (using a 1% solution)	No impact.	0
25	Ethyl acetate	40 ppm (using a 10% solution)	The monellin increases the volatility together with the sweet perception.	1
30	Acetaldehyde	5 ppm (using a 1% solution)	A higher, sweet impact; with no change in volatility.	1
35	Isobutyl thiazole (TOMATOZOLE®)	0.1 ppm (using a 0.1% solution)	The green vegetable tomato nuance impact is greatly increased. The natural tomato nuance is also improved.	5
40	Natural 2, 5-dimethyl pyrazine	1 ppm (using a 1% solution)	The pyrazine impact is increased. The naturalness of the product is increased.	2
45	Trimethyl pyrazine (TERRAZINE® 001)	1 ppm (using a 1% solution)	Pyrazine impact strengthened.	3
50	2-Methoxy-3-isobutyl pyrazine (GALBAZINE®)	0.01 ppm (using a 0.1% solution)	Pyrazine note is enhanced with a more natural impact. The taste can be described as "extremely powerful, earthy, herbaceous, vegetable, green bell pepper flavor" having use levels in the finished consumer product of from 0.0001-0.05 ppm.	3
55	Menthol	20 ppm (using a 10% solution)	Freshness, effervescent effect and fresh impact is improved.	4
	Trans-2-hexenal	1 ppm (using a 1% solution)	A green, fruity, stronger aroma and taste with waxy nuances.	1

(continued)

Molecule	Molecule Concentration	Evaluation	Quality Ranking on a Scale of 1-5, with 5 Being the Best of Overall Sensory Perception Value
Trans-2-hexenal	4 ppm (using a 1% solution)	A green aroma and taste with soapy nuance; but not "natural".	1
Natural isopulegol	2 ppm (using a 1% solution)	A minty, cooling aroma with a larger perception of cooling on the tongue.	3
Natural isopulegol	5 ppm (using a 1% solution)	The monellin causes a larger impact having a rounder flavor with a sweet, sugar background.	3
Trans-2-hexenol	2 ppm (using a 1% solution)	The monellin causes a real increase of intensity, a fuller greater impact on the side of the tongue.	4
6-Methyl-5-hepten-2-one	1 ppm (using a 1% solution)	The monellin increases the power of the methyl heptenone giving it a more natural impact.	4
Methyl acetophenone	2 ppm (using a 1% solution)	The monellin causes the product to be more natural and causes it to be a strong "living fruit" aroma and taste.	5
Vanilla extract	200 ppm	The monellin causes the material to have a richer vanilla aroma, more creamy with a fuller impact.	5
Vanillin	5 ppm (using a 1% solution)	A stronger impact with a sweet, sugar increase.	4
"Prenol" (3-methyl-2-buten-1-ol)	0.1 ppm (using a 0.1% solution)	The monellin causes the prenil to have a greater volatility and a "third" dimension is given to the green, fruity nuance with a more natural impact.	4
"Prenol" (3-methyl-2-buten-1-ol)	1 ppm (using a 1% solution)	The monellin gives a slight increase to the green, fruity aroma; but the best impact is at 0.1 ppm rather than at 1 ppm of prenil.	2
Mixture of 2 ppm Prenol and 10 ppm ethyl acetate	mixture (2 ppm and 10 ppm of each compound)	An enhanced, natural fruity note.	4

(continued)

	Molecule	Molecule Concentration	Evaluation	Quality Ranking on a Scale of 1-5, with 5 Being the Best of Overall Sensory Perception Value
5				
10	Mixture of 1 ppm prenol; 10 ppm ethyl acetate; and 5 ppm CITRAL	mixture (1 ppm prenol; 10 ppm ethyl acetate; and 5 ppm CITRAL )	The monellin gives to this mixture a greater lift and a real, fresh lemon juice nuance.	5
15	Peanut butter (reduced fat)	0.1 ppm monellin in peanut butter	A natural, rich, fresh roast peanut nuance with diminished, bitter, salty peanut skin nuance.	4
20	Peanut butter (reduced fat)	1 ppm monellin	A natural, fresh roast peanut nuance with diminished, acid, salty, bitter impact.	3
25	Natural banana essence (produced via pervaporation)	10 ppm (using a 10% solution)	The flavor impact is stronger with a more natural impression.	3
30	Peach essence (produced via pervaporation)	10 ppm (using a 10% solution)	The ethyl decanoate note is boosted causing the impact to be a "fatty" impact.	1
35	Ethyl decanoate	5 ppm (using a 1% solution)	The fatty, waxy impact is increased.	4
40	Raspberry essence (produced via pervaporation) (also Containing 10% $\alpha$ -terpineol)	10 ppm (using a 10% solution)	The impression of $\beta$ -ionone is vastly reduced; causing a real improvement with a natural, fresh, warm, fruity note.	5
45	$\beta$ -Ionone	3 ppm (using a 1% solution)	The monellin causes the ionone impact to be stronger and the chemical note is eliminated causing a real ripe, fresh, fruity, raspberry note to be created; cutting the floral impact and causing a natural fruit nuance to develop.	5
50	Bis(methylfuryl) disulfide	0.04 ppm (using a 0.001% solution)	The monellin causes the product to have a fuller, more juicy flavor with a natural roasted meat impact.	5
55	Farnesene	4 ppm (using a 1% solution)	The monellin causes the flavor impact to be natural.	5

(continued)

Molecule	Molecule Concentration	Evaluation	Quality Ranking on a Scale of 1-5, with 5 Being the Best of Overall Sensory Perception Value
Germacrene	4 ppm (using a 1% solution)	The natural nuance is vastly increased.	5
Ethyl pyruvate	5 ppm (using a 1% solution)	The natural impact is vastly increased.	5
1,3,5-Undecatriene	5 ppb (using a 0.001% solution)	The galbanum impact is vastly increased.	5
Natural indole	0.5 ppm (using a 0.1% solution)	The chemical taste is eliminated and a natural honeysuckle, LIVING FLOWER aroma and taste nuance is created using the monellin.	5

**BRIEF DESCRIPTION OF THE DRAWINGS****[0023]**

Figure 1 shows the amino acid sequence of the A- and B-chains of the natural monellin protein.

Figure 2 is a representation of the three-dimensional conformation of the natural, native monellin protein.

Figure 3A shows the amino acid sequence of a single-chain monellin protein analogue prepared according to the process of U.S. Letters Patent No. 5,487,983, and the nucleotide sequence of a synthetic gene useful in synthesizing the single-chain monellin protein.

Figure 3B(i) shows the amino acid sequence (leaving room for substitution at position 35 using the symbol "Xaa") of Japanese Published Application No. JP05/070494 (abstracted at *Chemical Abstracts*, Volume 119:48069g, entitled "Single-stranded monellins for improved thermostability", the contents of which abstract are incorporated herein by reference. The symbol "Xaa" represents either of glycine, serine, glutamic acid or threonine.

Figure 3B(ii) shows the amino acid sequence of a complete single-chain monellin protein of Published Japanese Application No. JP05/070494 abstracted at *Chemical Abstracts*, Volume 119:48069g.

Figure 4A sets forth the  $\alpha$ -carbon trace of the backbone of a single-chain monellin produced according to the process of U.S. Letters Patent No. 5,487,983 issued on January 30, 1996 with dots at the  $\alpha$ -carbon positions of residues that have been probed by mutagenesis.

Figure 4B sets forth the  $\alpha$ -carbon trace of the backbones of natural monellin with dots at the  $\alpha$ -carbon positions of residues that have been probed by mutagenesis.

Figure 5 is a liquid chromatogram profile for TALIN® (trademark of Tate and Lyle Limited of the United Kingdom), a mixture of Thaumatin I, Thaumatin II and Thaumatin B (conditions: S-Sepharose column operating at 7 ml per minute; gradient: 0-25mM NaCl (2 x 750 ml); fraction size: 45 ml).

Figure 6 is the high pressure liquid chromatography profile for the same TALIN® as set forth concerning the brief description of Figure 5.

**DETAILED DESCRIPTION OF THE DRAWINGS**

**[0024]** As stated at column 9, line 12 of U.S. Letters Patent No. 5,487,983, the protein of the amino acid sequence



shown in Figure 3A is encoded by a DNA sequence as they are shown. As shown in Figure 3A, nucleotides 1-141 encode residues 1-45 of the native B-chain preceded by a MET encoding ATG start code on, nucleotides 142-165 encode the linking "C" portion of eight amino acids, and nucleotides 166-285 encode residues 6-45 of the native A protein.

**[0025]** The protein of the monellin amino acid sequence shown in Figure 3B(ii) is also encoded by a DNA sequence as thereshown. As shown in Figure 3B(ii), the nucleotides shown thereon encode residues 1-95 of the single-chain preceded by a MET encoding ATG start code on. The amino acid sequence of Figure 3B(ii) is a replication of the amino acid sequence of Figure 3B(i) whereat at position 51, "Xaa" is replaced by a glycine ("Gly") moiety. As stated, supra, the "Xaa" protein at position 51 can be either a glycine, a serine, a glutamic acid or a threonine moiety and is produced according to the procedure of Japanese Published Application No. JPO5/070494 abstracted at *Chemical Abstracts*, Volume 119:48069g, which abstract is set forth as follows:

119: 48069g Single-stranded monellins for improved thermostability. Iijima, Hiroshi; Sone, Hidetaka (Kirin Brewery) Jpn. Kokai Tokkyo Koho JP 05 70,494 [93 70,494] (Cl. C07K7/10), 23 Mar 1993, JP Appl. 90/196,983, 25 Jul 1990; 29 pp. The single-stranded (ss) monellins (I) retain the sweetness of the wild type two-stranded I and have enhanced thermostability, esp. in lower pH, and protease resistance. The ss-I are prep'd. by linking the C-terminus of B chain of I with the N-terminal Phe of A chain with a linker amino acid Xaa (Xaa = Gly, Ser, Glu, or Thr). Prepn. of a ss-I using Gly as the linker amino acid by expression of the synthetic gene in *Escherichia coli* and *Saccharomyces cerevisiae* was shown. The ss-I retained its sweetness after incubation for 20 mins at 100°, but the wild type lost its sweetness at 50°. Moreover, the ss-I but not the wild type counterpart was resistant to proteinase degn. with e.g. *Achromobacter* proteinase I. Replacing the Val<sub>12</sub> or Val<sub>37</sub> of the ss-I with Ala did not alter the thermostability and protease resistance.

**[0026]** Referring to Figure 4A, the  $\alpha$ -carbon trace of the backbone of single-chain monellin, the numbered dots at the  $\alpha$ -carbon positions of residues that have been probed by mutagenesis correspond precisely to the numbers set forth for the amino acid sequence in Figure 3A with "N" corresponding to a methionine moiety, the number "2" being a glycine moiety, the number "3" being a glutamic acid moiety, the number "5" being a glutamic acid moiety, the number "6" being an isoleucine moiety, the number "7" being an isoleucine moiety, the number "8" being an aspartic acid moiety, the number "9" being an isoleucine moiety, the number "13" being a threonine moiety, the number "17" being a glycine moiety, the number "21" being a valine moiety, the number "25" being an asparagine moiety, the number "34" being a threonine moiety, the number "36" being an asparagine moiety, the number "42" being a cysteine moiety, the number "44" being a lysine moiety, the number "50" being an asparagine moiety, the number "54" being an isoleucine moiety, the number "57" being a tyrosine moiety, the number "59" being a tyrosine moiety, the number "64" being a tyrosine moiety, the number "66" being a serine moiety, the number "70" being a phenyl alanine moiety, the number "72" being an alanine moiety, the number "75" being a serine moiety, the number "76" being a glutamic acid moiety, the number "78" being a tyrosine moiety, the number "83" being an arginine moiety, the number "86" being a leucine moiety, the number "93" being a proline moiety; and the terminal, "C" being a proline moiety.

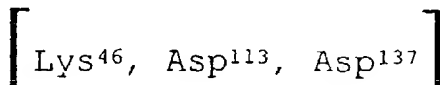
**[0027]** Figure 4B sets forth an  $\alpha$ -carbon trace of the backbones of the natural monellin protein with dots at the  $\alpha$ -carbon positions of the residues that have been probed by mutagenesis for each of two strands, a first strand beginning with N' and ending at C' and the second strand beginning at N" and ending at C". The strand beginning at N' and ending at C' corresponds to the B-chain of Figure 1. The strand beginning at N" and ending at C" corresponds to the A-chain of Figure 1.

**[0028]** With reference to the "A-chain", beginning at N" and ending at C", the dot indicated by N" is for methionine. Reference numeral 2' represents the moiety glycine. Reference numeral 3' represents the moiety glutamic acid. Reference numeral 5' represents the moiety glutamic acid. Reference numeral 6' represents the isoleucine moiety. Reference numeral 7' represents the isoleucine moiety. Reference numeral 8' represents the aspartic acid moiety. Reference numeral 9' represents the isoleucine moiety. Reference numeral 13' represents the threonine moiety. Reference numeral 17' represents the glycine moiety. Reference numeral 21' represents the valine moiety. Reference numeral 25' represents the asparagine moiety. Reference numeral 34' represents the threonine moiety. Reference numeral 36' represents the asparagine moiety. Reference numeral 42' represents the cysteine moiety. Reference numeral 44' represents the lysine moiety. Reference numeral 49' represents the glutamic acid moiety. Reference numeral 49A' which corresponds to the end of the chain at C" represents phenyl alanine.

**[0029]** With respect to chain-B, reference numeral 50' which corresponds to the beginning of the chain at N' represents arginine. Reference numeral 51' represents a glutamic acid moiety. Reference numeral 54' represents an isoleucine moiety. Reference numeral 57' represents a tyrosine moiety. Reference numeral 59' represents a tyrosine moiety. Reference numeral 64' represents a tyrosine moiety. Reference numeral 66' represents a serine moiety. Reference numeral 70' represents a phenyl alanine moiety. Reference numeral 72' represents an alanine moiety. Reference numeral 75' represents a serine moiety. Reference numeral 76' represents a glutamic acid moiety. Reference numeral 78' represents a tyrosine moiety. Reference numeral 83' represents an arginine moiety. Reference numeral 86' represents a leucine moiety. Reference numeral 93' represents a proline moiety. The end of the chain, C', is a proline moiety.

**[0030]** Figure 5 is a liquid chromatogram profile for TALIN® (trademark of Tate and Lyle Limited of the United King-

dom), a mixture of Thaumatin I, Thaumatin II and Thaumatin B (conditions: S-Sepharose column operating at 7 ml per minute; gradient: 0-25mM NaCl (2 x 750 ml); fraction size: 45 ml). The peak indicated by reference numeral 2316 is for that part of TALIN® which is known as "Thaumatin I" as described in U.S. Letters Patent No. 5,221,624 issued on June 22, 1993, the specification for which is incorporated by reference herein. The Thaumatin I can also be shown using the symbolism:



where "Lys" stands for "lysine" moiety; and "Asp" stands for an "Aspartic acid" moiety. The peaks indicated by reference numerals 2312 and 2314 are for "Thaumatin B" and "Thaumatin II" as described in United States Letters Patent No. 4,771,000, the specification for which is incorporated by reference herein.

[0031] The features disclosed in the foregoing description, in the following claims and/or in the accompanying drawings may both separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

## Claims

1. A consumable, ingestible fermentation-derived beverage or distilled spirit, alcoholic beverage composition comprising:
  - (i) from 1.5% up to 70% by weight of an alcohol or ester selected from the group consisting of ethyl alcohol, isoamyl alcohol, *n*-hexanol, *L*-menthol and cis-3-hexenyl acetate;
  - (ii) from 0.05 parts per million (ppm) up to 15 ppm of monellin or an analogue thereof; and
  - (iii) from about 0.1 ppb up to about 2% by weight of a fermentation-derived beverage or distilled spirit flavorant.
2. The composition of claim 1, wherein the fermentation-derived beverage or distilled spirit is a fruit liqueur.
3. The composition of claim 2, wherein the fruit liqueur is selected from blueberry liqueur, apple/pear liqueur or lemon liqueur.
4. The composition of any one of the preceding claims, wherein the fermentation-derived beverage or distilled spirit flavorant is selected from a blueberry flavor composition, a pear flavor composition, or a lemon flavor composition.
5. The composition of any one of the preceding claims, wherein the concentration of monellin or an analogue thereof is 10 ppm and the liqueurs are blueberry liqueur or liqueur Perle de Brillet.
6. The composition of any of the preceding claims also containing from 0.05 ppm up to 2% by weight of succinic acid.
7. The composition of any one of the preceding claims, also containing from 0.05 ppm up to 2% by weight of sclareolide.
8. The composition of any one of the preceding claims, also containing from 0.1 ppb up to 15 ppm of thaumatin.
9. A process for augmenting or enhancing the aroma and taste of a fermentation-derived beverage or distilled spirit comprising the step of adding to a fermentation-derived beverage or distilled spirit from 0.05 ppm up to 15 ppm of monellin or an analogue thereof.
10. The process of claim 9, wherein the fermentation-derived beverage or distilled spirit is a fruit liqueur.
11. The process of claim 10, wherein the fruit liqueur is pear liqueur.
12. A fermentation-derived beverage or distilled spirit containing from 0.05 ppm up to 15 ppm of monellin or an analogue thereof.

# Patentansprüche

1. Zusammensetzung eines konsumierbaren alkoholischen Getränkes, nämlich eines durch Fermentierung gewonnenen Getränkes oder eines alkoholischen Destillats, enthaltend

(i) von 1,5 Gew.-% bis zu 70 Gew.-% eines der folgenden Alkohole oder Ester, nämlich Ethylalkohol, Isoamylalkohol, n-Hexanol, L-Menthol oder cis-3-Hexenylacetat;

(ii) von 0,05 ppm bis zu 15 ppm Monellin oder eines seiner Analogen; sowie

(iii) von ungefähr 0,1 ppb bis zu ungefähr 2 Gew.-% eines Geschmacksstoffes für durch Fermentierung gewonnene Getränke oder alkoholische Destillate.

2. Zusammensetzung nach Anspruch 1, mit einem Fruchtlikör als ein durch Fermentierung gewonnenes Getränk oder alkoholisches Destillat.

3. Zusammensetzung nach Anspruch 2, mit einem Blaubeerlikör, einem Apfel/Birnen-Likör oder einem Zitronenlikör als Fruchtlikör.

4. Zusammensetzung nach einem der vorhergehenden Ansprüche, mit einem Blaubeeraroma-Geschmacksstoff, einem Birnenaroma-Geschmacksstoff oder einem Zitronenaroma-Geschmacksstoff als Geschmacksstoff für die durch Fermentierung gewonnenen Getränke oder alkoholischen Destillate.

5. Zusammensetzung nach einem der vorhergehenden Ansprüche, mit einer Konzentration von 10 ppm Monellin oder eines seiner Analogen in einem Blaubeerlikör oder in einem Liqueur Perle de Brillet.

6. Zusammensetzung nach einem der vorhergehenden Ansprüche, mit einem zusätzlichen Gehalt von 0,05 ppm bis zu 2 Gew.-% Bernsteinsäure.

7. Zusammensetzung nach einem der vorhergehenden Ansprüche, mit einem zusätzlichen Gehalt von 0,05 ppm bis zu 2 Gew.-% Sclareolid.

8. Zusammensetzung nach einem der vorhergehenden Ansprüche, mit einem zusätzlichen Gehalt von 0,1 ppb bis zu 15 ppb Thaumatin.

9. Verfahren zum Verbessern und Verstärken des Aromas und Geschmacks eines durch Fermentierung gewonnenen Getränkes oder eines alkoholischen Destillats mit der Verfahrensstufe des Zusetzens von 0,05 ppm bis zu 15 ppm Monellin oder eines seiner Analogen zu dem durch Fermentierung gewonnenen Getränk oder alkoholischen Destillat.

10. Verfahren nach Anspruch 9 mit einem Fruchtlikör als ein durch Fermentierung gewonnenes Getränk oder alkoholisches Destillat.

11. Verfahren nach Anspruch 10 mit einem Birnenlikör als Fruchtlikör.

12. Ein durch Fermentierung gewonnenes Getränk oder alkoholisches Destillat enthaltend von 0,05 ppm bis zu 15 ppm Monellin oder eines seiner Analogen.

## Revendications

1. Boisson comestible, dérivée de la fermentation, ingérable ou alcool distillé, la composition de la boisson alcoolisée comprenant :

(i) entre 1,5 % et 70 % en poids d'un alcool ou ester choisi dans le groupe constitué de l'alcool éthylique, l'alcool isoamylrique, le n-hexanol, le L-menthol et l'acétate de cis-3-hexenyl;

(ii) de 0,05 partie par million (ppm) à 15 ppm de monelline ou d'un de ses analogues ; et

(iii) de environ 0,1 parties par milliard à environ 2 % en poids d'une boisson dérivée de la fermentation ou essence d'alcool distillé.

- 5      2. Composition selon la revendication 1, dans laquelle la boisson dérivée de la fermentation ou alcool distillé est une liqueur de fruits.
3. Composition selon la revendication 2, dans laquelle la liqueur de fruits est choisie parmi la liqueur de myrtille, liqueur de pomme / poire ou liqueur de citron.
- 10     4. Composition selon l'une quelconque des revendications précédentes, dans laquelle la boisson dérivée de la fermentation ou essence d'alcool distillé est choisie parmi une composition d'essence de myrtille, une composition d'essence de poire ou une composition d'essence de citron.
- 15     5. Composition selon l'une quelconque des revendications précédentes, dans laquelle la concentration de la monelline ou d'un de ses analogues est 10 ppm, et les liqueurs sont de la liqueur de myrtille ou de la liqueur Perle de Brillet.
6. Composition selon l'une quelconque des revendications précédentes, contenant également de 0,05 ppm à 2 % en poids d'acide succinique.
- 20     7. Composition selon l'une quelconque des revendications précédentes, contenant également de 0,05 ppm à 2 % en poids de sclaréolide.
8. Composition selon l'une quelconque des revendications précédentes, contenant également de 0,1 ppb à 15 ppm de thaumatococine.
- 25     9. Procédé permettant d'augmenter ou améliorer l'arôme et le goût d'une boisson dérivée de la fermentation ou alcool distillé comprenant l'étape consistant à ajouter à une boisson dérivée de la fermentation ou un alcool distillé de 0,05 ppm à 15 ppm de monelline ou d'un de ses analogues.
- 30     10. Procédé selon la revendication 9, **caractérisé en ce que** la boisson dérivée de la fermentation ou alcool distillé est une liqueur de fruits.
11. Procédé selon la revendication 10, dans lequel la liqueur de fruits est une liqueur de poire.
- 35     12. Boisson dérivée de la fermentation ou alcool distillé contenant de 0,05 ppm à 15 ppm de monelline ou d'un de ses analogues.

40

45

50

55

AMINO ACID SEQUENCE OF NATURAL MONELLIN

A-Chain

1

\*PHE ARG GLU ILE LYS GLY TYR GLU TYR GLN  
LEU TYR VAL TYR ALA SER ASP LYS LEU PHE  
ARG ALA ASP ILE SER GLU ASP TYR LYS THR  
ARG GLY ARG LYS LEU LEU ARG PHE ASN GLY  
PRO VAL PRO PRO PRO

45

FIG.1

B-Chain

1

GLY GLU TRP GLU ILE ILE ASP ILE GLY PRO  
PHE THR GLN ASN LEU GLY LYS PHE ALA VAL  
ASP GLU GLU ASN LYS ILE GLY GLN TYR GLY  
ARG LEU THR PHE ASN LYS VAL ILE ARG PRO  
CYS MET LYS LYS THR ILE TYR GLU ASN GLU

50

\* PHE presents only in 10 % of natural monellin.

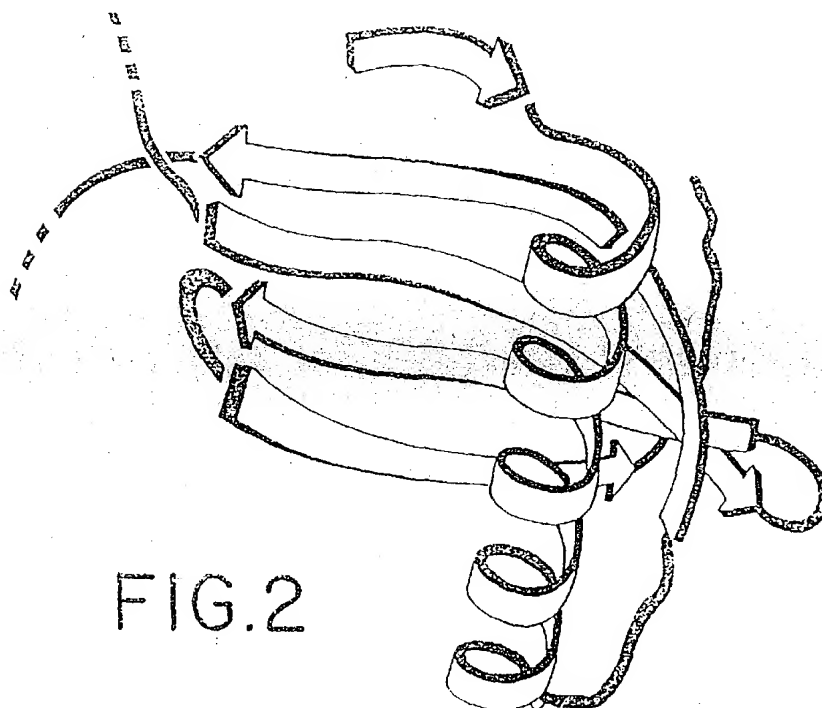


FIG.2

## FIG. 3-A

5'-	ATG	GGA	GGA	TGG	GAA	ATT	ATC	GAT	ATT	GGA	CCA	TTC	ACT	CAA	AAC	TTG	GGT	AAG	TTC	GCT
	Met	Gly	Gly	Trp	Glu	Ile	Ile	Asp	Ile	Gly	Pro	Phe	Thr	Gln	Asn	Leu	Gly	Lys	Phe	Ala
	GTT	GAC	GAA	GAA	AAG	AAG	ATT	GGT	GAA	TAT	GGT	AGA	TTG	ACT	TTC	AAC	AAG	GTT	ATT	
	Val	Asp	Glu	Glu	Lys	Lys	Ile	Gly	Gln	Tyr	Gly	Arg	Leu	Thr	Phe	Asn	Lys	Val	Ile	Arg
	CCA	TGT	ATG	AAG	AAG	ACT	ATT	TAC	GAA	AAC	GAA	AGA	GAA	ATT	AAG	GGG	TAC	GAA	TAC	CAA
	Pro	Cys	Met	Lys	Lys	Thr	Ile	Tyr	Glu	Asn	Glu	Arg	Glu	Ile	Lys	Gly	Tyr	Glu	Tyr	Gln
	TTG	TAT	GTT	TAC	GCT	TCT	GAC	AAG	CTT	TTC	AGA	GCT	GAC	ATT	TCT	GAA	GAC	TAC	AAG	
	Leu	Tyr	Val	Tyr	Ala	Ser	Asp	Lys	Leu	Phe	Arg	Ala	Asp	Ile	Ser	Glu	Asp	Tyr	Lys	Thr
	CGC	GGT	AGA	AAG	TTG	TTG	AGA	TTC	AAC	GGT	CCA	GTT	CCA	CCA	CCA	CCA				
	Arg	Gly	Arg	Lys	Leu	Leu	Arg	Phe	Asn	Gly	Pro	Val	Pro	Pro	Pro					

## FIG. 3-B(i)

Gly Glu Trp Glu Ile Ile Asp Ile Gly Pro Phe Thr Gln Asn Leu Gly  
 1 5 15  
 Lys Phe Ala Val Asp Glu Glu Asn Lys Ile Gly Gln Tyr Gly Arg Leu  
 20 25 30  
 Thr Phe Asn Lys Val Ile Arg Pro Cys Met Lys Lys Thr Ile Tyr Glu  
 35 40 45  
 Asn Glu Xaa Phe Arg Glu Ile Lys Gly Tyr Glu Tyr Gln Leu Tyr Val  
 50 55 60  
 Tyr Ala Ser Asp Lys Leu Phe Arg Ala Asp Ile Ser Glu Asp Tyr Lys  
 65 70 75 80  
 Thr Arg Gly Arg Lys Leu Leu Arg Phe Asn Gly Pro Val Pro Pro Pro  
 85 90 95

## FIG. 3-B(ii)

ATG	GGC	GAG	TGG	GAA	ATC	ATC	GAT	ATC	GGT	CCA	TTC	ACT	CAA	AAC	TTG
Met	Gly	Glu	Trp	Glu	Ile	Ile	Asp	Ile	Gly	Pro	Phe	Thr	Gln	Asn	Leu
1					5					10					15
GGT	AAA	TTC	GCT	GTT	GAT	GAA	GAA	AAC	AAG	ATT	GGC	CAA	TAC	GGT	AGA
Gly	Lys	Phe	Ala	Val	Asp	Glu	Glu	Asn	Lys	Ile	Gly	Gln	Tyr	Gly	Arg
				20					25					30	
TTG	ACC	TTT	AAC	AAG	GTT	ATC	AGA	CCA	TGC	ATG	AAG	AAG	ACT	ATT	TAC
Leu	Thr	Phe	Asn	Lys	Val	Ile	Arg	Pro	Cys	Met	Lys	Lys	Thr	Ile	Tyr
			35				40						45		
GAA	AAC	GAA	GGT	TTT	AGA	GAA	ATT	AAG	GGT	TAC	GAA	TAC	CAA	TTG	TAC
Glu	Asn	Glu	Gly	Phe	Arg	Glu	Ile	Lys	Gly	Tyr	Glu	Tyr	Gln	Leu	Tyr
			50				55					60			
GTA	TAC	GCT	TCT	GAC	AAG	TTG	TTC	CGT	GCT	GAC	ATT	TCC	GAA	GAC	TAC
Val	Tyr	Ala	Ser	Asp	Lys	Leu	Phe	Arg	Ala	Asp	Ile	Ser	Glu	Asp	Tyr
						70					75				
AAG	ACA	CGT	GGT	CGT	AAG	TTG	TTG	AGA	TTC	AAC	GGT	CCA	GTC	CCA	CCA
Lys	Thr	Arg	Gly	Arg	Lys	Leu	Leu	Arg	Phe	Asn	Gly	Pro	Val	Pro	Pro
80					85					90					95



FIG.4-A

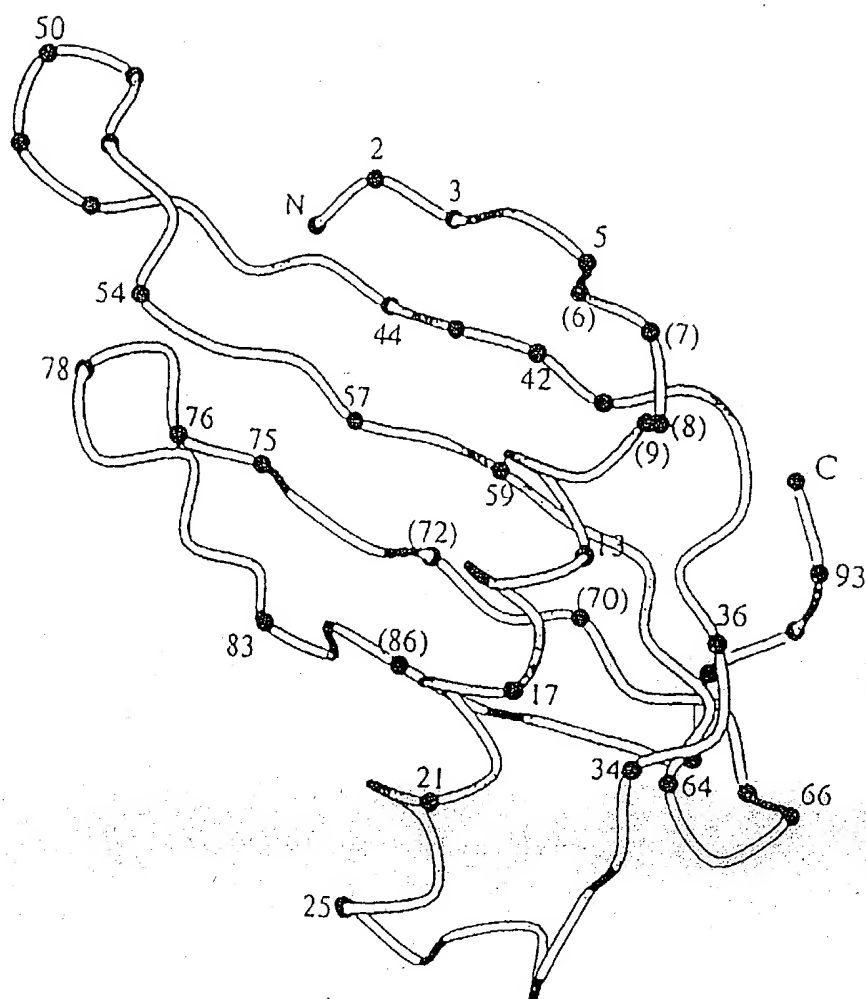


FIG.4-B

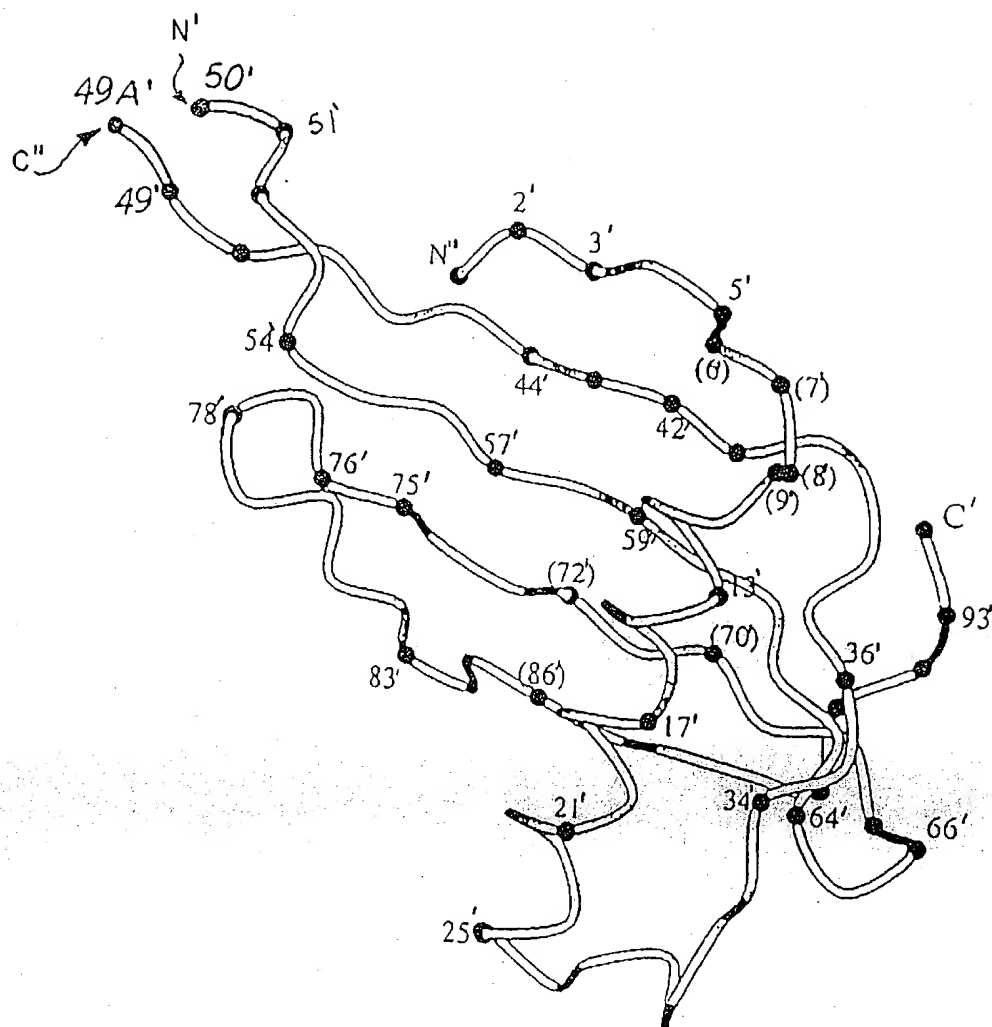


FIG. 5  
LC PROFILE

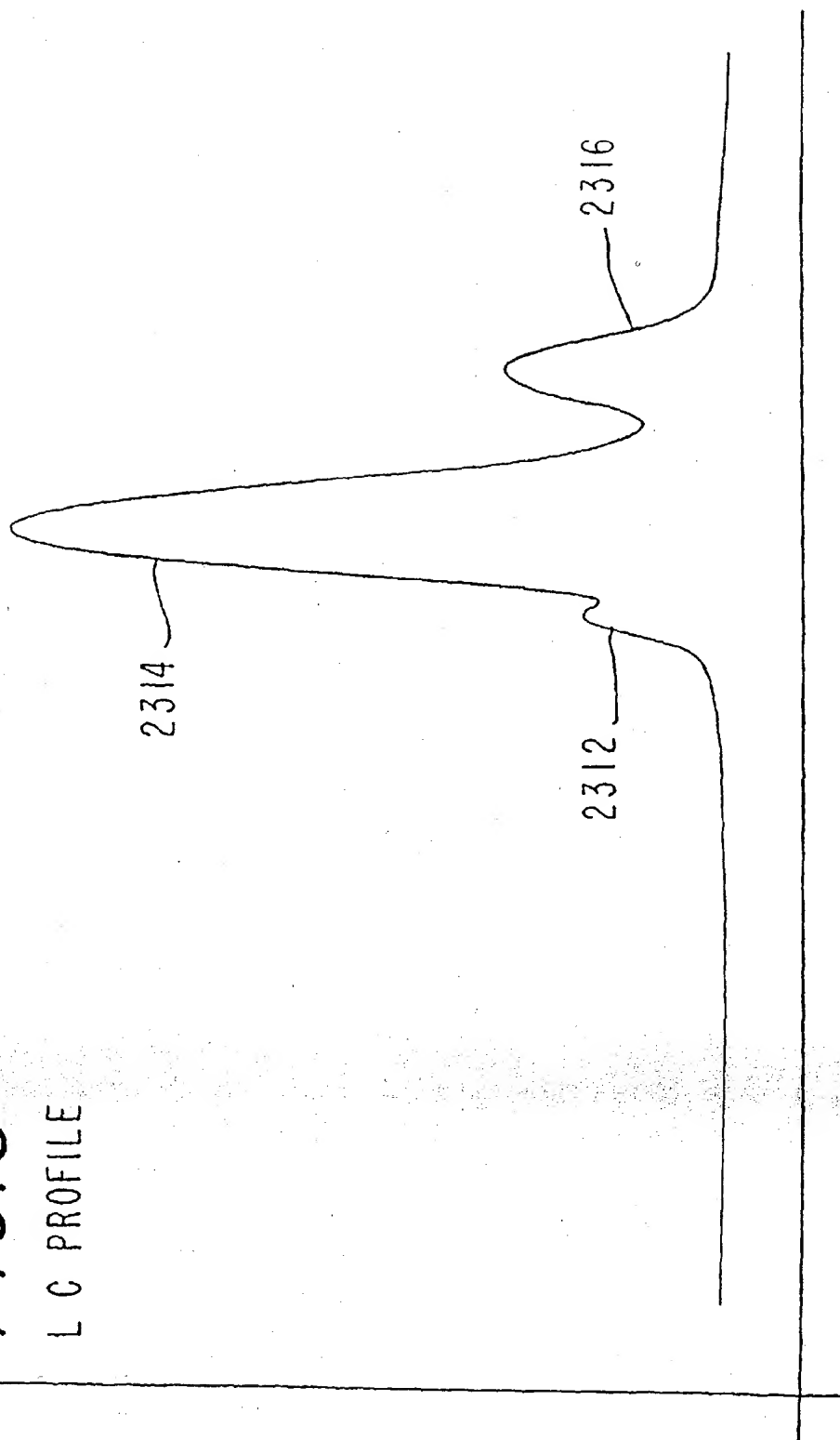


FIG.6

